

# Near-infrared reflectance spectroscopy for predicting lipid content in duck breast meat

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Abbreviated title: NIRS and meat lipid content

## Summary

Within the framework of a project requiring an important number of measures of lipid and water content in duck breast meat, near-infrared spectroscopy (NIRS) was evaluated in order to limit the use of the reference method requiring long analytical process. Three modes of spectra acquisition were tested: direct measurement on intact breast muscle just after the cutting and boning process (spectrometer ASD Labspec Pro) or later in the laboratory (spectrometer FOSS NIRS system) on ground meat or slices of breast meat. Calibration equations based on ground meat were the most precise. The SECV (standard error of cross-validation) was 0.31 % for lipids and 0.57 % for water, the repeatability of reference measurements being 0.23 % and 0.30 %, respectively. The data obtained on slices were less precise (lipids: SECV=0.51 %; water: SECV=0.73 %). The direct measurements on breast muscle also gave poorer calibrations (lipids: SECV=0.48 %; water: SECV=0.64 %). In conclusion, the prediction with NIRS of lipid and water content gave satisfactory data, particularly when realized in the laboratory on ground breast meat. Direct measurements were less precise, but allowed, nevertheless, a first valid estimation of the chemical composition.

Keywords: near-infrared spectroscopy, NIRS, duck, breast meat, lipid

## Introduction

The determination of meat chemical composition is necessary to control product quality in food-processing industries or in the frame of scientific studies concerning nutrition or genetic parameters. The most common measurements are water, lipid or protein content and sometimes more specific components such as fatty acids. These chemical analyses do not present much difficulty, but as soon as a large number of samples is concerned, they represent a lot of work, are costly, and require long delays before obtaining results. For example, only a few samples per day (about 10-20/day/person) can be tested with the reference method of lipid extraction from meat (Folch et al., 1957). If the number of samples is too large, the work becomes heavy or unfeasible.

Near-infrared reflectance spectrometry (NIRS) is an analytical method based on light absorption ( $\lambda=800-2500\text{nm}$ ) by organic material. The absorption level is related to the nature and quantity of chemical links and then to the chemical composition of product. After calibrating the equipment the measurement is easy and quick, allowing the prediction of chemical composition of hundreds of samples with a low cost. It is also possible to install online systems directly in the food-processing industries. This technique was yet tested to estimate meat quality (Prevolnik et al., 2004) in many cases, included chicken meat (Windham et al., 2003) or goose fatty liver (Molette et al., 2001).

The present study was conducted in the framework of a research project on genetic determinism of various duck traits (GENECAN, Marie-Etancelin et al., 2008) in which it was necessary to determine the chemical composition of about 1500 breast muscles of mule ducks. In the present methodological study, we compared different equipments and different methods for spectra acquisition in order to optimize the use of NIRS for the prospective applications.

## Materials and methods

### Samples

A total of 741 breast samples were used. The breasts were obtained in 2006 from mule ducks reared, overfed and slaughtered under industrial conditions at the INRA experimental unit of Artiguères (UEPFG, France). A first spectrometric measurement was done immediately after slaughter directly on the breast muscle (Pectoralis major).

Then on each breast a round slice (40 mm diameter) was removed and two more meat samples (about 20 g per sample) were ground. All these samples were stored at -20°C for further spectrometric and chemical analyses.

## **Spectrometric measurements**

Two spectrometers were used: a laboratory equipment FOSS NIRSystem 6500 (400-2500 nm) with a DCFA (Direct Contact Food Analysis) module, and portable equipment ASD Labspec Pro (350-2500 nm) with a « contact probe » module. All measurements were done in reflectance mode.

Three types of spectrometric measurement were done:

Direct measurement on breast muscle with the ASD spectrometer just after cutting and deboning carcass at 4°C, 24 h after slaughter. For each breast muscle, 8 spectra (4 measurement points on each breast repeated twice) were taken. The points were standardised (upper, right, left and down part of the internal side of breast).

Measurement with the FOSS spectrometer on ground breast muscle and presented in quartz cells. Each sample was measured three times (different cup filling) and spectra were averaged.

Measurement with the FOSS spectrometer on breast slices presented in quartz cells, with also three spectra readings.

## **Chemical analyses**

A subset of 130 samples was selected in order to represent the spectral variability of all ground samples measured with FOSS spectrometer. The selection was performed on the basis of the average distances (Mahalanobis distance H) between samples in a principal component analysis (PCA) on the spectra. These 130 samples were analysed with the reference laboratory methods: cold lipid extraction with chloroform-methanol (Folch *et al.*, 1957) and moisture (oven at 104°C until a constant sample weight). The analyses were done in duplicate on some samples in order to calculate the repeatability of the laboratory measurements.

## **Statistical analysis**

The spectral data were treated with WINISI software (Infrasoft Int., Port Matilda, PA, USA).

The visible wavelengths (400-800 nm) were not used in order to avoid too sensitive models taking into account colour differences not induced by chemical composition. The wavelengths presenting too much noise (>2200 nm for ASD and >2450 nm for FOSS) were also discarded from the analysis. The mathematical pre-treatment of spectra was determined in order to optimize the model performances. The optimal pre-processing was found to be the 1<sup>st</sup> derivative after normalization and smoothing on 10 measurements (WINISI SNVD procedure 1, 10, 5, 1). The calibration equations were developed by Partial Least Square (PLS) regression. The calibration performances were described by their coefficient of determination ( $R^2$ ), and their residual standard error of calibration (SEC) and cross validation (SECV). The ratio RPD = SD/SECV was calculated as a synthetic criterion of model quality.

## **Results and discussion**

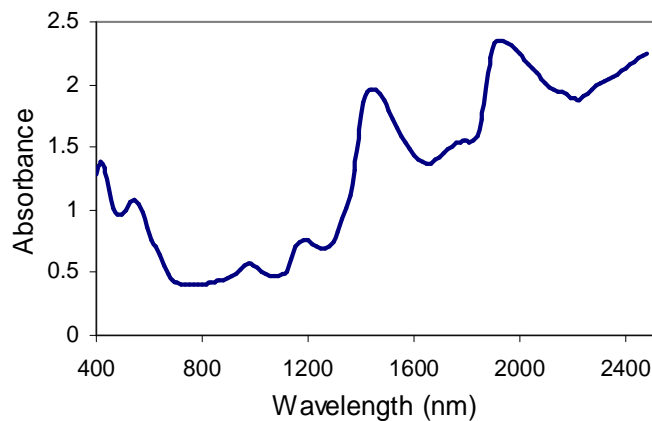
### **Laboratory measurements**

The repeatability of reference measurements was 0.23% for lipids and 0.30% for moisture. The average values and the standard deviation of measurements were  $5.1\% \pm 1.7$  for lipids and  $71.7\% \pm 1.3$  for moisture.

### **Spectra**

The spectra obtained with the two equipments had the same general aspect (Figure 1); they were characterized by high absorption peaks for water (980 nm, 1450 nm, 1950 nm). The databases were coherent; only 7 points (less than 1% of spectra) were considered as spectral outliers in the FOSS spectral basis. Moreover, the extreme spectral values were not the same for the two bases, suggesting more an artefact effect induced by spectral measurement than atypical samples.

Figure 1. Average spectra of duck breast muscle

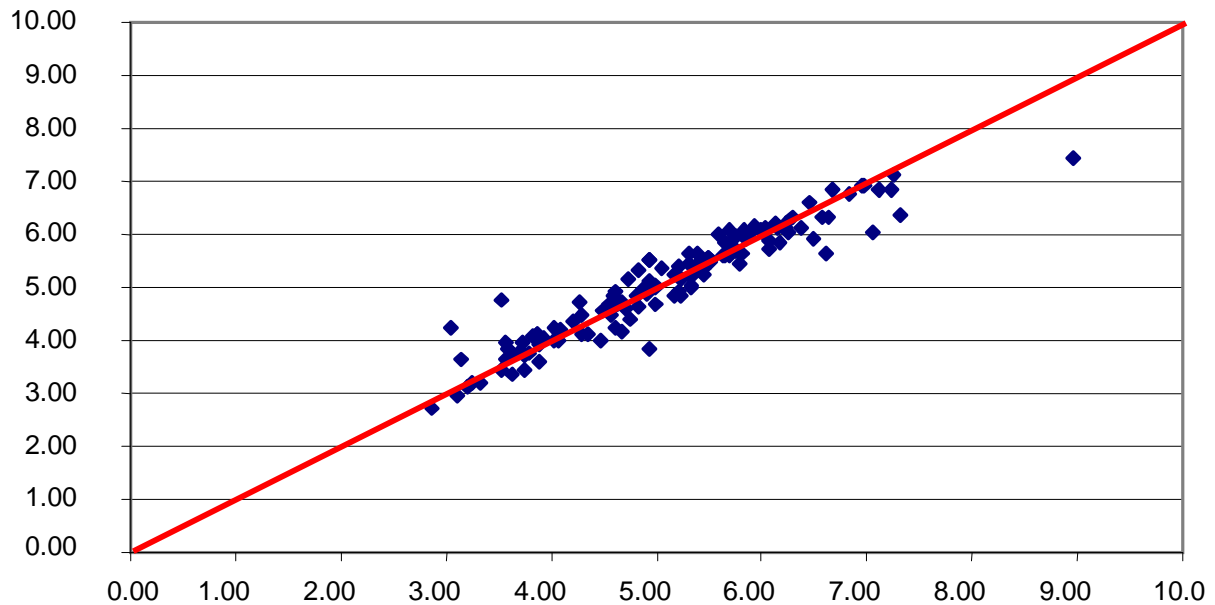


## FOSS calibration on ground samples

The most precise prediction for lipid content was obtained with FOSS spectra measured on ground samples. This result seems to be logical because these spectra were obtained under standardised conditions on homogeneous samples and similar to those used with the usual laboratory reference method. Under these conditions, the SEC was 0.25%, value similar to the repeatability obtained with the laboratory reference method (0.23%). The cross-validation (SECV) gave an error of 0.31%, which is acceptable for that type of measurement. Figure 2 shows the relationship between the predicted and measured lipid contents. Under similar conditions, Cozzolino *et al.* (1996) obtained SECV=0.54% in chicken meat. Berzaghi *et al.* (2005) obtained 0.24% but with a relatively low number of samples. For moisture level, the model obtained with spectrometer FOSS had a SEC = 0.51% and a SECV = 0.57%, which is two times higher than the repeatability calculated with the reference laboratory method. The relative weakness of RPD (2.3) was due to the low variability of the database. The precision of moisture level measurement also depended on the storing conditions of samples. Spectral measurements were done with thawed samples; therefore free water could interfere with NIRS measurements.

However the calibration performances obtained for moisture in our study were a bit better than those reported by Cozzolino *et al.* (1996, 0.70%) but lower than those from Berzaghi *et al.* (2005, 0.19% for dry matter) on chicken meat.

Figure 2. Prediction of lipid content in ground samples by using FOSS calibration



## Other procedures of spectra aquisition

The spectra obtained on breast slices with FOSS spectrometer gave less precise calibrations than those determined with ground samples. The SECV was 1.6 and 1.3-fold higher for lipids and moisture respectively and the RPD values were much lower. This result was probably due to the fact that the slices represented a limited part of breast, whereas the chemical analysis was realised on samples more representative of the whole muscle. Moreover the surface of slice (presence of water) probably affected spectral measurement. Cozzolino *et al.* (1996) showed higher effects of condition measurements on calibration models with SECV increasing from 0.54 to 0.90% for lipids and from 0.70 to 1.59% for moisture.

The spectra measured directly on breast muscles, just after carcass cutting with ASD spectrometer also gave less precise calibrations than those obtained with FOSS spectrometer on ground samples. The SECV obtained for lipids was similar to that obtained with FOSS spectrometer on muscle slices and 1.5-fold higher than that obtained with ground samples. For moisture, the performances were nearest to those obtained on ground samples then 1.1-fold higher for SECV. Both the equipments and the measurement conditions were different. In this case, the moisture estimation was less affected than that of lipids probably because the direct measurement avoided the freezing and thawing steps.

The ASD spectra were done on four measurement points defined on the internal side of breast muscle. Calibrations equations were calculated for each place in order to determine the best one. The data presented on Tables 1 and 2 shows that the average spectra from 8 measurements was always more precise than each single place. It is concluded that the number of spectra is more important than their location on the muscle.

Table 1. Calibration equations for lipid level

	SEC	R <sup>2</sup>	SECV	RPD
FOSS spectrometer				
<i>Ground sample</i>	0.25	0.94	0.31	3.3
<i>Breast slice</i>	0.43	0.84	0.51	2.1
ASD spectrometer				
<i>All points (average)</i>	0.44	0.84	0.48	2.3
<i>Point 1</i>	0.46	0.82	0.51	2.1
<i>Point 2</i>	0.50	0.77	0.56	1.9
<i>Point 3</i>	0.47	0.80	0.52	2.0
<i>Point 4</i>	0.41	0.86	0.48	2.3

Table 2. Calibration equations for moisture level

	SEC	R <sup>2</sup>	SECV	RPD
FOSS spectrometer				
<i>Ground sample</i>	0.51	0.85	0.57	2.3
<i>Breast slice</i>	0.55	0.82	0.73	1.7
ASD spectrometer				
<i>All points (average)</i>	0.60	0.77	0.64	2.0
<i>Point 1</i>	0.67	0.74	0.72	1.8
<i>Point 2</i>	0.68	0.72	0.72	1.8
<i>Point 3</i>	0.67	0.73	0.71	1.8
<i>Point 4</i>	0.70	0.71	0.76	1.7

## Validation elements

For a unique series of data, the cross validation is a good tool to evaluate calibrations. A true validation requires new independent samples.

In the present study some elements allowed to evaluate the pertinence of estimated lipid levels in breast meat. With the calibrations obtained from the 130 samples analysed according the laboratory reference method (Folch *et al.*, 1957), all the 741 individuals from the experiment were predicted. The predictions obtained with FOSS and ASD spectrometers were compared. The correlation between these two prediction was not high ( $R^2=0.75$ ) because the relationship cumulated the errors on both figures (errors were independent,  $p>0.05$ ). However, the slope was close to 1 and the bias was weak, showing the coherence between distinct spectra measurements. Another comparison element was the relationship between lipids and water. The relationship between these two parameters for the reference data was:

$$\text{Water}_{\text{ref}} = 76.69 - 0.979 \times \text{LIP}_{\text{ref}} \quad (R^2 = 0.65, n = 130).$$

For the predicted data the relationship was almost similar:

$$\text{Water}_{\text{SPIR}} = 76.70 - 0.989 \times \text{LIP}_{\text{SPIR}} \quad (R^2 = 0.72, n = 741),$$
 suggesting that predicted data respected the biological logic.

A second series of 735 animals were raised in 2007, with the same requirement for chemical composition analysis. The analysis is in progress, but preliminary results suggest that NIRS prediction is still valid but require the update of the calibration equation with a subset of reference analysis, since the direct use of the 2006 equation for 2007 samples leads to some prediction biases.

## CONCLUSION

This study confirmed the feasibility of NIRS measurement to determine moisture and lipid levels in duck breast muscle. It allowed to specify the optimal measurement conditions and showed that the predictions obtained with ground samples in the laboratory were better than those determined directly on breast muscles in the slaughterhouse. However, the measurements on the whole breast muscles with portable equipment gave calibrations with a reasonable precision. The spectra measurement with ASD equipment is fast and does not require any sample preparation. Therefore this method can be interesting for practical applications under industrial conditions. The prediction equations allowed predicting the lipid and moisture levels for 741 duck breast muscles from our experiment. The use of the equation for other series of samples requires an update of the equation, but with only a limited number of samples analysed for reference analyses.



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